REMARKS

STATUS OF THE CLAIMS

Claims 16-29 were pending in this application. Claims 16 and 22 have been amended. Claim 30 has been added. Claims 28 and 29 have been cancelled without prejudice. Following entry of the amendments claims 16-28 and 30 will be pending and at issue.

SUPPORT FOR AMENDMENTS TO CLAIMS

Claim 16 was amended to recite "wherein said dsRNA is non-autocomplementary." Support for this claim amendment is found in prior claim 28 (now cancelled) and throughout the specification, such as at p. 27-32, which makes clear that there are two embodiments, one that is autocomplementary and one that is formed from two *separate* strands, and so is non-autocomplementary ("A region II which is complementary within the double-stranded structure is formed by two separate RNA single strands or by autocomplementary regions of a topologically closed RNA single strand which is preferably in circular form.").

Claim 22 was amended to correct a minor typographical error.

New claim 30 recites "wherein inhibiting the expression of said target gene further comprises inhibiting expression at a concentration of the dsRNA that is lower by one order of magnitude than a concentration required for a corresponding single-stranded oligoribonucleotide to inhibit expression." Support for this new claim is found throughout the instant specification as filed, including at p. 7, lines 28-33 and p. 8, lines 22-28.

<u>Priority Applications</u>: Corresponding support for the claim amendment and new claim can be found at the same page and line numbers in priority applications U.S. Patent Application No. 10/612,179, U.S. App. No. 09/889,802 (filed on September 17, 2001), and PCT WO00/44895 (PCT/DE00/00244; filed on January 29, 2000).

The amendment and new claim are believed not to introduce new matter, and its entry is respectfully requested.

PRIORITY

The Examiner acknowledged filing of all of the certified priority documents and certified translations. Thus, Applicants maintain that the claims are entitled to a priority date of at least January 29, 2000.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claims 16-29 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Specifically, the Examiner stated that there is no support for "dsRNA-mediated interference," indicating that the support provided by Applicants in the prior response is "taken out of context" or is merely a recitation of "the work of others." *See* Office Action, p. 3. Applicants respectfully disagree.

Upon reading the entire section of the specification from page 1, line 20 to page 3, line 25, it is clear that the phrase "via dsRNA-mediated interference" is supported. Starting at page 1, lines 20-32, the specification presents the disadvantages of antisense technology, including that "antisense RNA must be introduced into the cell in an amount which is at least as high as the amount of the mRNA," and that "known antisense methods are not particularly effective." On page 2, lines 11-21, the specification goes on to describe Fire's solution to the disadvantages of the previous art (e.g., antisense art), which is a dsRNA that inhibits expression of a nematode gene "highly efficiently." Regarding Fire's principle of operation, the specification explains that "it is believed that particular activity of the dsRNA used in nematode cells is not due to the antisense principle but possibly on catalytic properties of the dsRNA, or enzymes induced by it." Specification, page 2, lines 11-21. The specification notes also that, while Fire described inhibiting expression of a nematode gene, Fire did not describe inhibiting gene expression in "mammalian and human cells." *Id.* The specification then proceeds to explain that the object of the claimed invention is to "do away with the disadvantages of the prior art" (e.g., antisense art, Fire's dsRNAs that are only described with regard to nematode cells, etc.). *Id.* at p. 2, lines 23-24. Specifically, the invention includes dsRNAs that cause "particularly effective inhibition" (like Fire), but also operate in "mammalian and human cells" (unlike Fire). *Id.* at p. 2, lines 23-29; p.3, lines 10-25; *see also* p. 7, lines 28-33 and p.

8, lines 22-28 ("Compared with the use of single-stranded oligoribonucleotides, the inhibition is already caused at concentrations which are lower by one order of magnitude when using dsRNA."). The specification further explains that, "surprisingly," they have discovered that this "effective inhibition" occurs even when the complementary region of the dsRNA is "not more than 49 base pairs in length" (unlike Fire, which referred to much longer dsRNAs). *Id.* at p. 3, lines 10-25; p. 8, lines 10-13. It indicates that, in mammalian and human cells, the dsRNAs over 50 nucleotides in length induce dsRNA-dependent protein kinase that "leads to the disappearance of the interference effect mediated by the dsRNA," and so the invention "overcomes this disadvantage in particular" (i.e., allows the dsRNA-mediated interference effect to occur). *Id.* at p. 3, lines 17-25. In addition, the specification states that, "in comparison with the use of single-stranded oligoribonucleotides

The Examiner quoted page 3, lines 17-25 (paragraph 11) of the specification (cited by the Applicant in the prior response as support for dsRNA-mediated interference), stating that this was taken out of context and does not provide support. However, Applicant submits that one of ordinary skill in the art would have clearly understood this paragraph, in the context of all of the surrounding paragraphs, to say that the invention operates via dsRNA-mediated interference (similar to Fire), including such operation in mammalian and human cells (unlike Fire, which is one of the "disadvantages" that the invention will "do away with"). One of ordinary skill in the area of dsRNA art would have been thoroughly familiar with the work of Fire and his discovery of dsRNA-mediated interference (later coined RNAi by Fire) by long dsRNAs in nematodes, for which Fire later won the Nobel Prize. *See* Fire, et al., *Potent and Specific Genetic Interference by double-stranded RNA in Caenorhabditis elegans*, Nature 391:806-810 (1998) ("...effects of dsRNA-mediated interference are potent and specific...."). One of ordinary skill in the art would have also been familiar with the state of the dsRNA art, in general, at the time of filing (at least as early as Jan. 2000), and would have been aware that Fire's dsRNA-mediated interference effect was known not to occur in mammals or humans. So, one of ordinary skill would have excitedly noted the specification's description of shorter dsRNAs (e.g., less than 49 base pairs, which includes 21-nucleotide dsRNAs) that surprisingly provide this "effective inhibition" in mammals and humans, overcoming the disadvantage posed by longer dsRNAs (over 50 nucleotide pairs) that induce the protein kinase that

normally causes the dsRNA-mediated interference to disappear in mammals and humans. This was an important leap forward in the dsRNA-mediated interference world at the early date of (at least) January 2000, and this point in the specification surely would not have been missed or misunderstood by one of ordinary skill in the dsRNA art at that time.

Accordingly, Applicants submit that there is support in the specification for dsRNA-mediated interference. Thus, Applicants respectfully request withdrawal of this ground of rejection.

DOUBLE-PATENTING REJECTIONS

Claims 2, 4-6, 9, 11 and 32-40 are provisionally on the ground of nonstatutory obviousness-type double-patenting over certain claims of U.S. Application Nos. 11,982,425, 11/982,345, 11/982,434, 11/982,441, 10/382,395, and 11/982,305. As the Examiner acknowledged, Applicant requests that the rejections be held in abeyance until allowable subject matter is indicated in the instant claims.

REJECTIONS UNDER 35 U.S.C. § 103

Claims 16-29 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Tuschl et al. (WO 02/44321), Bernstein et al. (Nature 2001) and Gao et al. (Nucleic Acids Research 1995). Applicants respectfully submit that the claimed invention is entitled to a priority date of January 29, 2000. Thus, Tuschl and Bernstein are not available as prior art against the claimed invention. Thus, withdrawal of this rejection is respectfully requested.

Claims 16-29 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Agrawal et al. (WO 94/01550), Baracchini et al. (US 5,801,154), Bennett et al. (US 5,703,054), and Gao et al. (Nucleic Acids Research 1995). Applicant traverses this ground of rejection.

Independent claim 16 recites the following (emphasis added):

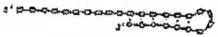
> 16. An isolated oligoribonucleotide consisting of **two separate complementary RNA single strands forming a double-stranded structure (dsRNA),**
> wherein said separate RNA strands are chemically linked,
> **wherein said dsRNA is non-autocomplementary**,
> wherein the dsRNA is 21 base pairs in length,
> wherein the dsRNA does not comprise a full length RNA transcript of a mammalian target gene,
> wherein one strand of the dsRNA is complementary to less than the full length of an RNA transcript of said mammalian target gene, and

wherein the dsRNA specifically inhibits the expression of said mammalian target gene using dsRNA-mediated interference.

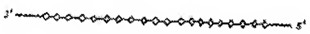
A. Two Separate Strands forming Double-Stranded Structure (dsRNA)

The Examiner notes that Applicant argued that Agrawal does not teach two separate RNA single strands that are chemically linked, as claimed. Office Action, p. 12-13. However, the Examiner states that Agrawal's "use of a non-nucleotide linker would make the self-complementary region and hybridizing region two separate complementary nucleic acid strands and this would meet the limitations of the instant claims." *Id.* Applicant respectfully disagrees. The section of Agrawal cited by the Examiner makes clear that the non-nucleotide linker connects two *regions* of a single antisense strand rather than connecting two separate *strands*, and these *regions* are referred to throughout Agrawal's description. *See* Agrawal, p. 15, starting at line 31; Abstract ("The invention provides antisense oligonucleotides…that are called self-stabilized oligonucleotides and comprise two *regions*") (emphasis added). In addition, the self-stabilized oligoribonucleotide of Agrawal would not be *self*-stabilized if it were stabilized by forming a duplex with a separate strand. In fact, as the Agrawal figure reproduced below shows, even after the self-stabilized oligoribonucleotide (I) hybridizes to the target molecule (II), the hybridized form (III) is still a single strand that has unfolded itself, and the self-complementary region (small circles) remains connected to the target hybridizing region (small rectangles).

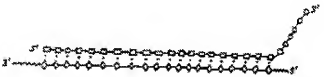
I.



II.



III.



In addition, Agrawal teaches away from the claimed invention reciting two separate strands. Agrawal describes the "self-stabilized oligonucleotides" throughout his disclosure, even entitling the application "SELF-STABILIZED OLIGONUCLEOTIDES AS THERAPEUTIC AGENTS." Agrawal further emphasizes throughout the importance of the two regions (rather than two strands) of the self-stabilized oligonucleotides. *Id.* at p. 5, lines 13-17 ("...the advantages of oligonucleotides according to the invention, known as *self-stabilized* oligonucleotides, *arise from the presence of two structural features: a target hybridizing region and a self-complementary region*.") (emphasis added). Given the clear importance of having these oligonucleotides be *self-stabilized* oligonucleotides with two *regions*, one of ordinary skill in the art would not have modified Agrawal's oligonucleotides to create the claimed dsRNA consisting of two separate strands for inhibiting expression.

B. dsRNA is non-autocomplementary

Agrawal does not teach "wherein said dsRNA is non-autocomplementary," as claimed. The specification explains that the dsRNA can be 1) "formed by two separate RNA single strands" or 2) formed by self-complementary or "autocomplementary regions." Specification, p. 4, lines 27-32. Claim 16 is drawn to description 1), as is further made clear by the language referring a non-autocomplementary dsRNA. Agrawal does not teach this claim limitation. Even in the section cited by the Examiner reciting a non-nucleotide linker, Agrawal specifically states that it has a <u>*self*</u>-complementary (or <u>*auto*</u>complementary) region, and the linker connects two <u>*regions*</u> (not strands). *See* Agrawal, p. 15, lines 31-35 ("In one preferred embodiment the <u>*self*</u>-complementary <u>*region*</u> may be connected to the target hybridizing <u>*region*</u> by a suitable non-nucleic acid linker.") (emphasis added). Agrawal does not introduce this linker as being in a non-<u>*self*</u>-stabilized embodiment. To the contrary, he introduces this in the paragraph following his paragraph introducing the <u>*self*</u>-complementary region of the <u>*self*</u>-stabilized oligonucleotides. *See id.* p. 15, lines 1-3. There is no suggestion that the non-nucleotide linker is included in anything other than a <u>*self*</u>-stabilized (or <u>*auto*</u>complementary) oligonucleotide. In addition, given Agrawal's teaching throughout of the importance of the two <u>*regions*</u> (rather than two strands) of the <u>*self*</u>-stabilized oligonucleotides, it would not have been obvious to combine Agrawal with

any references reciting non-autocomplementary oligonucleotides. *Id.* at p. 5, lines 13-17. Thus, Agrawal does not teach the claim limitation regarding a dsRNA that is non-autocomplementary.

C. dsRNA-Mediated Interference

The Examiner also states that "because the dsRNA is substantially identical to the claimed dsRNA, the dsRNA inherently possesses the functional characteristics of the claimed dsRNA, namely inhibitions of expression of a mammalian target using dsRNA-mediated interference." Office Action, p. 14. Applicant respectfully disagrees. As stated above, Agrawal does not teach a dsRNA formed of two separate RNA single strands, so Agrawal's self-stabilized oligonucleotides are not "substantially identical to the claimed dsRNA." Furthermore, MPEP 2112(V) states that the "PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [or her] claimed product." Applicant respectfully submits that Agrawal's oligonucleotides do not "necessarily or inherently possess the characteristics" of the claimed invention for multiple reasons.

First, Agrawal explains throughout his application that his oligonucleotides operate via antisense mechanisms. Antisense mechanisms were well known to be different from and inferior to dsRNA-mediated interference mechanisms. *See* Montgomery MK, Xu S, Fire A., *RNA as a target of double-stranded RNA-mediated genetic interference in Caenorhabditis elegans*, Proc. Natl. Acad. Sci. 95(26):15502-7, p.15502 (1998) ("...the potency of dsRNA effects in *C. elegans* has surprised us; in certain cases only a few molecules of dsRNA per cell are required to achieve effective knock-out....The substoichiometric activity of dsRNA indicates that RNAi in *C. elegans* cannot work by a traditional antisense mechanism: there is not enough antisense RNA in the injected material to bind stoichiometrically to the endogenous mRNA.") (also refers to "dsRNA-mediated interference" in Abstract); *see also* "RNA interference" in Wikipedia, last paragraph ("In investigating the regulation of muscle protein production, they observed that neither mRNA nor antisense RNA injections had an effect on protein production, but double-stranded RNA successfully silenced the targeted gene."). In addition, the specification makes clear that "compared with the use of single-stranded oligoribonucleotides" (e.g., antisense strands), the claimed dsRNAs inhibit expression at concentrations that are "lower by one order

of magnitude," suggesting that the dsRNA-mediated interference mechanisms are both different from and superior to the antisense mechanisms (see new claim 30, also not taught by Agrawal). Specification, p. 7, lines 28-33 and p. 8, lines 22-28. Thus, Agrawal's self-stabilized antisense oligonucleotides would not necessarily or inherently have specifically inhibited expression of a mammalian target gene using dsRNA-mediated interference.

Second, Agrawal's single, self-stabilized oligoribonucleotide unfolds with the two regions still attached in order to hybridize with a target molecule. This is different what is known to occur in dsRNA-mediated interference. In dsRNA-mediated interference, the short dsRNAs (also called siRNAs) will be "unwound into two ssRNA, namely the passenger strand and the guide strand. The passenger strand will be degraded, and the guide strand is incorporated into the RNA-induced silencing complex (RISC)." *See* "RNA interference" in Wikipedia. Then, "the guide strand base pairs with a complementary sequence of a messenger RNA molecule and induces cleavage…." *Id.* In contrast, even after Agrawal's self-stabilized oligoribonucleotide hybridizes to the target molecule, Agrawal shows that the hybridized form (item III in Figure shown above) is still a single strand that has unfolded itself (not the two separate strands of claim 16 that are chemically linked with e.g., labile linkage of claim 21 or other types of linkage claimed). The self-complementary region (small circles) remains connected to the target hybridizing region (small rectangles). This would not necessarily or inherently have resulted in dsRNA-mediated interference. In fact, this extra tail in Agrawal may necessarily prevent dsRNA-mediated interference from occurring. Further, if Agrawal's two regions were each 21 nucleotides in length and if it unfolded as shown in Agrawal's figures, the extra tail would result in an oligonucleotide of 42 nucleotides in length. Given the sensitivity regarding length of dsRNAs in mammals due to PKR responses, etc., this additional length may prevent dsRNA-mediated interference. Thus, Agrawal's self-stabilized antisense oligonucleotides would not necessarily or inherently have specifically inhibited expression of a mammalian target gene using dsRNA-mediated interference.

D. Secondary Considerations

In addition, Agrawal does not render the claimed invention obvious because of various secondary considerations that point to nonobviousness. *See Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966)

1. Long-felt but unsolved need

There was a long-felt but unsolved need in the art for an invention involving a dsRNA that would specifically inhibit expression of a mammalian target gene via dsRNA-mediated interference. At the time of the invention (e.g., at least as early as Jan. 2000), dsRNA-mediated interference had been found to operate in various organisms, including nematodes, fruit flies, planaria, plants, etc. However, though various articles indicated the desirability of dsRNA-mediated interference in mammals, articles as late as 2001 show that this need was still unsolved. *See* Tuschl T. et al., *Targeted mRNA degradation by double-stranded RNA in vitro*, Genes Dev. 15;13(24):3191-7, p. 3195 (Dec. 1999) ("Mouse cell lines lacking dsRNA-induced anti-viral pathways…may be useful in the search for mammalian RNAi. If RNAi exists in mammals, as might be predicted from the presence of RNAi-like phenomena in invertebrates, plants, and fungi…"); Clemens, et al., *Use of double-stranded RNA interference in Drosophila cell lines to dissect signal transduction pathways*, Proc. Natl. Acad. Sci. 97(12): 6499–6503, p. 6503 (June 6, 2000) ("The discovery that RNAi functions in *Drosophila* cell culture will undoubtedly aid in the elucidation of the mechanism that allows dsRNA to inhibit target protein synthesis and may serve as a stepping stone for adaptation of this technique to mammalian systems."); Elbashir et al., *RNA interference is mediated by 21- and 22-nucleotide RNAs*, Genes Dev. 2001 15: 188-200 ("The siRNAs may be effective in mammalian systems, where long dsRNAs cannot be used because they activate the dsRNA-dependent protein kinase (PKR) response…"). Based on the numerous statements from 1998 to 2001 regarding finding a way to inhibit expression of target genes in mammals using RNAi, this was a long-felt, recognized, and persistent need that was not solved by others, and that remained unsolved long after Agrawal published.

2. Failure of others

Others had been searching for a dsRNA that would specifically inhibit expression of a mammalian target gene via dsRNA-mediated interference, yet had failed. *See* section above entitled "Long-felt but unsolved need;" *see also* George Sen and Helen Blau, *A Brief History of RNAi: The Silence of the Genes*, FASEB J., 20:1293-1299, p. 1294 (2006) ("Up to this point [after January 2000], the use of RNAi was limited to flies, worms, and plants, as the introduction of long dsRNA into mammalian cells elicits an interferon response that causes a general inhibition of translation abrogating the specificity of RNAi. The finding that short dsRNA could silence genes heralded the use of RNAi in mammalian cells.").

3. Unexpected results

The results of the claimed combination were unexpected. The fact that a dsRNA that could specifically inhibit expression of a mammalian target gene via dsRNA-mediated interference was unexpected. At the time of filing, various articles indicated that dsRNA-mediated interference did not operate in mammals. *See* Fire, *RNA-trigged gene silencing*, Trends Genet. 15 (9) 358-363, p. 363 (1999) ("Mammals have a vehement response to dsRNA, the best-characterized component of which is a protein kinase (PKR) that responds to dsRNA by phosphorylating (and inactivating) translation factor EIF2a."). The specification stated that it was a *surprising* result that the shorter dsRNAs would specifically inhibit expression via dsRNA-mediated interference in mammals. *See* specification, p. 3, lines 10-25; p. 8, lines 10-13. Other art at the time confirmed that this was a surprising result. *See* George Sen and Helen Blau, *A Brief History of RNAi: The Silence of the Genes*, FASEB J, 20:1293-1299, p. 1294 (2006) ("The finding that short dsRNA could silence genes heralded the use of RNAi in mammalian cells."); Elbashir et al., *RNA interference is mediated by 21- and 22-nucleotide RNAs*, Genes Dev. 2001 15: 188-200 (noting with uncertainty, even as late as 2001, that "The siRNAs *may be* effective in mammalian systems, where long dsRNAs cannot be used because they activate the dsRNA-dependent protein kinase (PKR) response…." (emphasis added)). Thus, the results of the claimed combination were unexpected since it has the unexpected property of inhibiting expression of a mammalian target gene via dsRNA-mediated interference. *Id.* ("Presence of a property not possessed by the

prior art is evidence of nonobviousness."). In addition, it did not exhibit the expected PKR response described by art in the field with regard to mammals. *See* MPEP 716.02(a) ("Absence of property which a claimed invention would have been expected to possess based on the teachings of the prior art is evidence of unobviousness.").

Barancchini, Bennett, and Gao do not remedy any of the above-stated deficiencies in Agrawal. The Examiner relies on Barancchini only for oligoribonucleotides that are 8 to 30 or 12-25 nucleotides in length. *See* Office Action, pp. 7-8. The Examiner relies on Bennett only for oligonucleotides that can be up to 50 or 12-25 nucleotides in length. *See id.* The Examiner relies on Gao only for chemical linkage in a duplex structure. *See id.* Barancchini, Bennett, and Gao, alone or in combination, do not teach the elements described above as missing from Agrawal, nor does the Examiner argue that they do.

Accordingly, Agrawal, Barancchini, Bennett, and Gao do not disclose each and every element of the independent claim, nor the claims that depend therefrom. Thus, withdrawal of this rejection is respectfully requested.

CONCLUSION

Withdrawal of the pending rejections and reconsideration of the claims are respectfully requested, and a notice of allowance is earnestly solicited. If the Examiner has any questions concerning this Response, the Examiner is invited to telephone Applicant's representative at (650) 335-7185.

Respectfully submitted,

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By: / Antonia L. Sequeira /

Antonia L. Sequeira, Esq.
Reg. No.: 54,670
Fenwick & West LLP
Silicon Valley Center
801 California Street
Mountain View, CA 94041
Tel.: (650) 335-7185
Fax.: (650) 938-5200